# 201-15824B



**ROBUST SUMMARY**
**OF INFORMATION ON**

**Substance Group** **GREASE THICKENERS**

**Summary prepared by** American Petroleum Institute

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

### 1.1.1 GENERAL SUBSTANCE INFORMATION

**Substance type** : Petroleum product
**Physical status** : Solid

**Remark** : Lubricating greases are solid or semi-solid materials made by thickening lubricating oils with soaps.
The soaps are formed in-situ in the lubricating oil by the chemical reaction of an alkali and the respective fatty acid.
This robust summary covers the calcium and lithium greases in which calcium and lithium soaps respectively have been used as the thickening agent.

Information on several greases as well as on lithium stearate, a soap commonly used as a thickener, is included in this robust summary.
In addition some information is included on magnesium stearate (closely related to calcium stearate) and on castor oil (mostly ricinoleic acid) which is closely related to the larger fatty acids used to make the salts in this category.

09.09.2004

### 1.13 REVIEWS

**Memo** : Leonard et al

**Remark** : Leonard et al reviewed the available information on the teratogenicity, mutagenicity and carcinogenicity of lithium compounds.
Their conclusions were:
"Such effects would be highly unlikely in an occupational setting but might be a risk to the considerable percentage of the population treated for manic depressive disorders.
It was concluded that lithium compounds have no significant clastogenic and, based on studies in microorganisms, only doubtful mutagenic activity.
Information on teratogenic effects is contradictory. While some observations in man and a few animal studies suggest that lithium in concentrations in the order of those given to patients may cause malformations, other observations do not support this claim and the risk with carefully controlled therapy is probably small. .....
No information is available on cancer caused by treatment with lithium, and it is highly unlikely that lithium is carcinogenic."

24.12.2003 (14)

**Memo** : Cosmetic ingredient review panel

**Remark** : A cosmetic ingredients review panel concluded that stearate compounds are safe as cosmetic ingredients.

23.12.2003 (4)

### 2.1 MELTING POINT

**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The melting point estimates given here are for fatty acid salts covering this range of carbon atoms. The data represent a potential melting point range for all substances in the grease thickeners category.

**Result** :

| Molecular Weight | No. C Atoms | Estimated MP Value, (°C) |
|---|---|---|
| **Lithium Salts** | | |
| nonanedioic acid, dilithium salt | | |
| 200.09 | 9 | 186 |
| octadecanoic acid, lithium salt | | |
| 290.42 | 18 | 249 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | | |
| 306.42 | 18 | 264 |
| docosanoic acid, lithium salt | | |
| 346.53 | 22 | 271 |
| Calcium Salts | | |
| octadecanoic acid, 12-hydroxy, calcium salt | | |
| 639.03 (1) | 36 | 320 |
| stearic acid, calcium salt | | |
| 607.04 (1) | 36 | 288 |

(1) Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Estimated melting points were calculated using a validated computer model.

09.09.2004 (32)

### 2.2 BOILING POINT

**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The boiling point estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential boiling point range for all substances in the grease thickeners category.

**Result** :

| Molecular Weight | No. C Atoms | Estimated BP Value, (°C) |
|---|---|---|
| **Lithium Salts** | | |
| nonanedioic acid, dilithium salt | | |
| 200.09 | 9 | 484 |
| octadecanoic acid, lithium salt | | |
| 290.42 | 18 | 578 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | | |
| 306.42 | 18 | 611 |
| docosanoic acid, lithium salt | | |
| 346.53 | 22 | 624 |
| **Calcium Salts** | | |
| octadecanoic acid, 12-hydroxy, calcium salt | | |
| 639.03 (1) | 36 | 730 |
| stearic acid, calcium salt | | |
| 607.04 (1) | 36 | 661 |

(1) Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Estimated boiling points were calculated using a validated computer model.

09.09.2004 (32)

## 2.4 VAPOUR PRESSURE

**Decomposition** :
**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The vapor pressure estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential vapor pressure range for all substances in the grease thickeners category.

**Result** :

| Molecular Weight | No. C Atoms | Estimated VP Value, (hPa) |
|---|---|---|
| **Lithium Salts** | | |
| nonanedioic acid, dilithium salt | | |
| 200.09 | 9 | $2 \times 10^{-9}$ |
| octadecanoic acid, lithium salt | | |
| 290.42 | 18 | $1 \times 10^{-12}$ |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | | |
| 306.42 | 18 | $2 \times 10^{-16}$ |
| docosanoic acid, lithium salt | | |
| 346.53 | 22 | $5 \times 10^{-14}$ |
| Calcium Salts | | |
| octadecanoic acid, 12-hydroxy, calcium salt | | |
| 639.03 (1) | 36 | $1 \times 10^{-2}$1 |
| stearic acid, calcium salt | | |

607.04 [1] 36 $6 \times 10^{-14}$
[1] Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Estimated vapor pressures were calculated using a validated computer model.

17.11.2004 (32)

### 2.5 PARTITION COEFFICIENT

**Method** : Calculated by KOWWIN, V1.66 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : Because fatty acids are ionizable compounds, Kow measurements (hence log P) can vary greatly with pH. The variation depends upon pH and the pKa of the compound. In general, Kow values of a compound are lower when it exists predominantly in the ionized form as compared to existing primarily in the non-ionized form. The KOWWIN V1.66 model handles ion pairs in a special way and gives Kow estimates that are an estimate for the ionized acid. Many fatty acids have pKa values circumneutral, and they would exist predominantly in the molecular form at environmentally relevant pHs. Therefore, the estimates given here are potentially lower than what would be expected for the salt form at typical environmental pHs.

**Result** :

| | **Molecular Weight** | **No. C Atoms** | **Estimated Log Kow** |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | -3.56 |
| octadecanoic acid, lithium salt | 290.42 | 18 | 4.13 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | 306.42 | 18 | 2.60 |
| docosanoic acid, lithium salt | 346.53 | 22 | 6.10 |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | 639.03 [1] | 36 | 11.7 |
| stearic acid, calcium salt | 607.04 [1] | 36 | 14.3 |

[1] Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Estimated partition coefficients were calculated using a validated computer model.

17.11.2004 (32)

### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

**Solubility in** : Water
**Method** : Calculated by WSKOWWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**Test substance** : other TS: Grease thickeners

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The water solubility estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential water solubility range for all substances in the grease thickeners category.

**Result** :

| Molecular Weight | No. C Atoms | Estimated Solubility, mg/l |
|---|---|---|
| **Lithium Salts** | | |
| nonanedioic acid, dilithium salt | | |
| 200.09 | 9 | $1 \times 10^{6}$ |
| octadecanoic acid, lithium salt | | |
| 290.42 | 18 | 4.1 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | | |
| 306.42 | 18 | 0.1 |
| docosanoic acid, lithium salt | | |
| 346.53 | 22 | 0.04 |
| **Calcium Salts** | | |
| octadecanoic acid, 12-hydroxy, calcium salt | | |
| 639.03 (1) | 36 | $9.7 \times 10^{-9}$ |
| stearic acid, calcium salt | | |
| 607.04 (1) | 36 | $8.2 \times 10^{-11}$ |

(1) Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Water solubility estimates were calculated using a validated computer model.

09.09.2004 (32)

### 2.14 ADDITIONAL REMARKS

**Memo** : Physico-chemical properties of grease thickeners

**Remark** : Greases are formed through a chemical reaction of a mineral oil, a fatty acid, and a metal caustic (typically calcium or lithium hydroxide). This reaction occurs in the mineral oil matrix when a fatty acid or its methyl ester is dissolved in the mineral oil followed by the addition of the caustic. The caustic and fatty acid molecules react to form an insoluble metal salt of the fatty acid. Because the thickener is synthesized in situ during the manufacture of the finished grease, secondary interactions between the fatty acid salt and the mineral oil matrix also result, creating the physical consistency of grease (see also Section 1.1.1). The byproducts of this reaction are either water or methanol depending on whether the fatty acid or its methyl ester, respectively, was used as the reactant. When fatty acids are reacted with caustic outside of a mineral oil matrix, the resulting compounds are called soaps (NLGI, 1996).

Computer predictions for melting point, boiling point, vapor pressure, partition coefficient, and water solubility were made for the salts as if they existed outside the grease matrix. However, the endpoint values should be qualified with the understanding that the thickening agent is created by a chemical reaction in situ and does not exist as a separate entity outside of the grease matrix.

09.09.2004 (17)

### 3.1.1 PHOTODEGRADATION

**INDIRECT PHOTOLYSIS**

**Sensitizer** : OH
**Method** : Calculated by AOPWIN V1.90 (EPIWIN V3.10; EPA 2000)
**Year** : 2000
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : Due to the extremely low vapor pressure of these substances plus the fact that these compounds are made within a mineral oil matrix, there is essentially no opportunity for these substances to enter the atmosphere. However, the modeling results show that if any vapors entered the atmosphere, these molecules would undergo indirect photolysis reactions and not persist.

**Result** : Concentration of sensitizer: $1.5 \times 10^6$ OH/cm³

See table of half-lives below (values given in days):

| Test Substance | Molecular Weight | No. C Atoms | Estimated Half-life, (days) |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | 1.4 |
| octadecanoic acid, lithium salt | 290.42 | 18 | 0.5 |
| octadecanoic acid, 12- hydroxystearate, lithium salt | 306.42 | 18 | 0.4 |
| docosanoic acid, lithium salt | 346.53 | 22 | 0.4 |
| **Calcium Salts** | | | |
| octadanoic acid, 12-hydroxy, calcium salt | 639.03 | 36 (1) | 0.2 |
| stearic acid, calcium salt | 607.04 | 36 (1) | 0.2 |

(1) Composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
The endpoint was estimated using a validated computer model.

17.11.2004 (31)

### 3.1.2 STABILITY IN WATER

**GLP** : No
**Test substance** : Grease thickeners various

**Remark** : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components that comprise the grease thickener category are salts of fatty acids that are not subject to hydrolysis because they lack functional groups that hydrolyze.

**Reliability** : (1) valid without restriction
17.11.2004 (9)

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

**Method** : Calculations by Level 1, Version 2.02, a fugacity-based environmental equilibrium partitioning model (Mackay 1991).

**Remark** : Grease thickening agents are created by a chemical reaction within a mineral oil matrix resulting in the formation of grease. The distribution estimates given here are for pure compounds representing the range of molecular weights of substances in the grease thickeners category. When these substances exist in their pure state, fugacity modeling showed them to partition mostly to either soil or water. The degree of partitioning to either of these environmental compartments was related to the water solubility of the compound. These estimates should be used with the knowledge that such thickening agents are entrained within a grease matrix and such entrainment would limit environmental exposure.

**Result** :

Air, Water, Soil, Sediment, Suspended Sediment, Fish.

**PERCENT DISTRIBUTION**

| Number C Atoms | Air | Water | Soil | Sed. | Susp. Sed. | Fish |
|---|---|---|---|---|---|---|
| **Lithium Salts** | | | | | | |
| nonanedioic acid, dilithium salt | | | | | | |
| 9 | <0.1 | 100 | <0.1 | <0.1 | <0.1 | <0.1 |
| octadecanoic acid, lithium salt | | | | | | |
| 18 | <0.1 | 8 | 90 | 2 | <0.1 | <0.1 |
| octadecanoic acid, 12-hydroxy-, lithium salt | | | | | | |
| 18 | <0.1 | 73 | 26 | 0.6 | <0.1 | <0.1 |
| docosanoic acid, lithium salt | | | | | | |
| 22 | <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |
| **Calcium Salts** | | | | | | |
| octadecanoic acid, 12-hydroxy-, calcium salt | | | | | | |
| 18 | <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |

stearic acid, calcium salt
18 <0.1 <0.1 98 2 <0.1 <0.

**Reliability** : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model. The estimates given are for pure substances and not likely to reflect the disposition from a grease matrix.

17.11.2004 (16)

## 3.5 BIODEGRADATION

**Remark** : See Section 3.8
17.11.2004

## 3.8 ADDITIONAL REMARKS

**Memo** : Biodegradability of grease and grease thickeners

**Remark** : In order to assess the biodegradability of grease and grease thickeners, it is necessary to have an understanding of the components and manufacture of greases. As described in Section 1.1.1, the principle components making up grease are 1) mineral oil base fluid, 2) alkali metals such as lithium or calcium hydroxides, and 3) various fatty acids. When these individual components are combined in their proper proportions, the mineral oil thickens due to formation of the thickener (i.e., calcium or lithium salts of the fatty acids) and the affinity of the thickener for the base oil (NLGI, 1996). Proportions of the different reactants vary, but thickeners typically contribute 1% to 14% by weight, with the balance being made up of mineral oil and performance additives. Some residual water is generally present (approximately 10% of the thickener, or 0.1% to 1.4%) and indeed necessary for uniform dispersion of the thickener in the oil (NLGI, 1996).

Attempts to produce environmentally friendly greases that are biodegradable have focused primarily on alternatives for the mineral base oil (Grives, 1999; Faci, et al., 2003; Stempfel and Baumann, 2003). With base oil being greater than 65% of greases, it comprises a major component affecting biodegradability of the product. As described in the lubricating base oil HPV test plan and robust summaries (API, 2003), mineral base oils are not particularly amenable to biodegradation and would not be classified as readily biodegradable. In ready biodegradation testing, these substances degraded from 1.5% to 29% when tested by the OECD 301B procedure and 31% to 50% when tested by the OECD 301F method (API, 2003).

Faci et al. (2003) compared the biodegradation potential of mineral oil grease with one that had been formulated with vegetable oil. Using the OECD 301F method, biodegradation of the grease formulated with vegetable oil ranged from 62% to 75%, whereas the mineral oil grease achieved 5% to 8% biodegradation. The type of thickener used in the Faci et al. (2003) study was not specified, but Grives (1999) evaluated the biodegradability of vegetable oil-based greases thickened by inorganic clay with two preparations of a lithium hydroxystearate thickener. That study

found essentially no difference in biodegradation of vegetable oil greases prepared with the inorganic thickener (75% biodegradation) to those thickened with the organic fatty acid soap (75% and >85% biodegradation).

The thickeners in and of themselves would not be expected to persist in the environment except as part of the grease matrix. This is because they are preparations of fatty acids that are derived from edible animal fats or vegetable oils. Included in this category are stearic acid (C18), 12-hydroxystearic acid (C18), docosanoic acid (C22), hydrogenated castor oil (comprised of ricinoleic and similar acids, C18), and methyl esters of oxidized hydrocarbon waxes (=C18). One lithium salt of a dicarboxylic acid (azelaic, C9) is included in the category as it is commonly used in lithium complex greases. Azelaic acid is manufactured from ricinoleic acid (castor oil). The following biodegradation data include various analogs of some of the fatty acids in this category. These data show that fatty acids similar to those used in grease thickeners may be considered readily biodegradable or at least inherently biodegradable. . Fatty acids undergo aerobic biodegradation by the process of beta-oxidation. Beta-oxidation of the parent fatty acid forms acetate and a new fatty acid of two less carbon atoms. This process repeats itself until the compound is completely broken down. The hydrocarbon will eventually be degraded to $CO_2$ and $H_2O$ (Atlas and Bartha, 1993). For this reason, the length of the fatty acid chain does not preclude biodegradation, but it may take longer to achieve complete mineralization. The beta-oxidation sequence does not necessarily require the presence of molecular oxygen, and fatty acid biodegradation may proceed under anaerobic conditions (Atlas and Bartha, 1993).

Substances in the grease thickeners category are composed of calcium or lithium salts of fatty acids. These fatty acids range in size from 9 to 22 carbon atoms in length and represent substances of plant and animal origin. The following biodegradation data are intended to serve as surrogate estimates of the biodegradation potential of these grease thickeners.

| Substance | No. C atoms | Biodeg % | Method | Source |
|---|---|---|---|---|
| Surrogates for >C18 Fatty Acid Salts (CAS 4499-91-6; 68603-11-2) | | | | |
| Docosanoic Acid CAS# 112-85-6 | | | | |
| | 22 | 48 - 56 | OECD 301C | SIDS (2001) |
| | | 79 - 96 | OECD 302C | |
| Surrogates for C16-C18 Fatty Acid Salts (CAS 3159-62-4; 4485-12-5; 5342-16-5; 64754-95-6; 68783-36-8; 7620-77-1; 1592-23-0; 64755-01-7) | | | | |
| Sodium Stearate | | | | |
| | 18 | 89 | Modified Sturm | P&G Chemicals (2003) |
| Tall Oil CAS# 8002-26-4 | | | | |
| | 16 - 18 | 60 | OECD 301D | Pine Chemical Association (2001) |
| | | 73 | OECD 301F | |
| Tall Oil Fatty Acids CAS# 61790-12-3 | | | | |
| | 16 - 18 | 56 | OECD 301D | Pine Chemical |

| | | | | |
|---|---|---|---|---|
| | | | | Association (2001) |
| | | 84 | OECD 301F | |
| | | 74 | OECD 301C | |

Fatty Acids, C16-18 unsaturated branched & linear CAS# 68955-98-6

| | | | |
|---|---|---|---|
| 16 - 18 | 67 | EPA OPPTS 853.110 | Pine Chemical Association (2001) |

Tall Oil Fatty Acids, K-salt
CAS# 61790-44-1

| | | | |
|---|---|---|---|
| 16 - 18 | 79 | EPA OPPTS 853.110 | Pine Chemical Association (2001) |

Surrogates for C9 Fatty Acid salt (CAS No. 38900-29-7)

No data available. Biodegradation is expected to achieve similar rates to longer chained fatty acids via beta-oxidation metabolic pathway (see technical discussion).

03.12.2004 (1) (2) (5) (8) (17) (24) (25) (26) (30)

## 4.1 ACUTE/PROLONGED TOXICITY TO FISH

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Oncorhynchus mykiss (Fish, fresh water) |
| **Exposure period** | : | 7 day(s) |
| **Analytical monitoring** | : | No |
| **Method** | : | Reference method for determining acute lethality of effluents to rainbow trout. Environment Canada, EPS 1/RM/13 |
| **Year** | : | 2003 |
| **GLP** | : | No |
| **Test substance** | : | Grease with calcium soap thickener and performance additives |
| **Result** | : | There was 30% fish mortality in the grease treatment and 10% mortality in the control after 7 days. Control and treatment fish group weights were 5.8 and 6.0 g, respectively. |
| **Test condition** | : | Test substance was prepared for testing by spreading 250 g onto 20.3 cm x 25.3 cm glass sheet at a thickness of 1.0 cm.<br>The sheet was placed into a 22 L plastic pail with polyethylene liner and filled with 20 L of dechlorinated tap water as dilution water (loading rate = 12,500 mg grease/l).<br>Dilution water chemistry was not provided in the test report. A test vessel containing only dilution water was used as a control. A separate control with dilution water and an empty glass sheet was also used. There was one replicate per treatment. Ten fish were added to each test vessel (loading density <0.5 g/l) and fish survival was monitored daily for 7 days. At the end of the exposure period, the test fish were weighed. All treatments were pre-aerated at 6.5 ml/min/l. Temperature, pH, conductivity, and dissolved oxygen in the test vessels were monitored daily. During the test, temperature ranged from 14 to 15 °C, pH was 7.9 to 8.0, conductivity was 393 to 457 µS/cm, and dissolved oxygen was 8.6 to 9.1 mg/l. Fish used in testing were obtained from Ackenberry Trout Farms and held 22 days before testing. Fish mortality 7 days before test was <2%.<br>Control fish length ranged from 3.0 to 4.9 cm and fish weight ranged from 0.5 to 0.9 g. |
| **Reliability** | : | (2) valid with restrictions<br>Acceptable study following Environmental Canada test method and conducted by laboratory that is accredited by the Canadian Association of Environmental and Analytical Laboratories. Test report contained sufficient documentation except for dilution water quality parameters. Study lacked analytical monitoring of the test solution and only one unreplicated treatment was used. |

11.01.2005 (13)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Oncorhynchus mykiss (Fish, fresh water) |
| **Exposure period** | : | 7 day(s) |
| **Analytical monitoring** | : | No |
| **Method** | : | Reference method for determining acute lethality of effluents to rainbow trout. Environment Canada, EPS 1/RM/13 |
| **Year** | : | 2003 |
| **GLP** | : | No |
| **Test substance** | : | Grease with mixed calcium 12-hydroxystearate and tallow thickener and performance additives |
| **Result** | : | There was no fish mortality in the grease treatment and the control after 7 |

days. Control and treatment fish group weights were 4.5 and 5.6 g, respectively.

**Test condition** : Test substance was prepared for testing by spreading 250 g onto 20.3 cm x 25.3 cm glass sheet at a thickness of 1.0 cm. The sheet was placed into a 22 L plastic pail with polyethylene liner and filled with 20 L of dechlorinated tap water as dilution water (loading rate = 12,500 mg grease/l).
Dilution water chemistry was not provided in the test report. A test vessel containing only dilution water was used as a control. A separate control with dilution water and an empty glass sheet was also used. There was one replicate per treatment. Ten fish were added to each test vessel (loading density <0.5 g/l) and fish survival was monitored daily for 7 days. At the end of the exposure period, the test fish were weighed. All treatments were pre-aerated at 6.5 ml/min/l. Temperature, pH, conductivity, and dissolved oxygen in the test vessels were monitored daily. During the test, temperature ranged from 14 to 15 °C, pH was 7.7 to 8.0, conductivity was 412 to 458 µS/cm, and dissolved oxygen was 8.8 to 9.2 mg/l. Fish used in testing were obtained from Ackenberry Trout Farms and held 22 days before testing. Fish mortality 7 days before test was <2%.
Control fish length ranged from 3.2 to 4.8 cm and fish weight ranged from 0.3 to 0.9 g.

**Reliability** : (2) valid with restrictions
Acceptable study following Environmental Canada test method and conducted by laboratory that is accredited by the Canadian Association of Environmental and Analytical Laboratories. Test report contained sufficient documentation except for dilution water quality parameters. Study lacked analytical monitoring of the test solution and only one unreplicated treatment was used.

11.01.2005 (13)

## 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

**Type** : Static
**Species** : Acartia tonsa
**Exposure period** : 48 hour(s)
**Analytical monitoring** : No
**Method** : MAFF/U.K.OCNS/PARCOM
**Year** : 1994
**GLP** : Yes
**Test substance** : Grease with Calcium 12-hydroxystearate (CAS No. 3159-62-4) thickener and performance additives

**Result** : 48-h $EL_{50}$ >1000 mg/l WAF.
Immobilization in the 1000 mg/l WAF of the grease was 13% after 48 h. Control immobilization was 3%. Exposure of A. tonsa to the 1.0 mg/l 3,5-DCP solution resulted in 34% (32 organisms tested) immobilization. Numbers of immobilized A. tonsa were 1, 4, and 11 in the control, 1000 mg/l WAF, and 3,5-DCP reference, respectively.
The temperature range recorded during the test was 0.2 °C outside the recommended levels of 18 to 22 °C. This deviation was not considered significant.

**Test condition** : A 1000 mg/l water accommodated fraction was prepared by stirring 2 g of the grease in 2 L of artificial seawater for 24 h. The solution was allowed to stand for at least 1 h before the aqueous phase was drawn off and used undiluted as the test medium. Artificial seawater was prepared by dissolving artificial seasalts (Tropic Marin, Aquatechnik,

Wartenberg, W. Germany) in reverse osmosis purified water to a salinity of 34 ± 2o/oo. Triplicate groups with 10 A. tonsa were exposed to 100 ml of test solution held in 200 ml glass crystallizing dishes. Three dishes containing artificial seawater only served as controls. Three dishes containing 1 mg/l solution of a reference compound 3,5-dichlorophenol (DCP) were also prepared. Aged A. tonsa (23 days old) from laboratory cultures were used. Original stocks were supplied by the Vandkvalitetsinstituttet, Copenhagen, Denmark.
Immobilized A. tonsa were recorded and removed from test vessels after 24 and 48 h. At the end of the test a few drops of formalin were added to the test vessels to preserve the organisms for subsequent counting. Test was carried out in temperature controlled room set at 20 ± 2 °C with a 16h light, 8h dark illumination cycle. Test solutions were not renewed or aerated during test. Salinity ranged from 34 to 35o/oo. pH ranged from 8.2 to 8.3. Dissolved oxygen was 7.2 to 7.6 at 0 h and 6.8 to 7.0 at 48 h. Temperature throughout the test was 17.8 to 19.3 °C.

**Reliability** : (2) valid with restrictions
Only one concentration of the grease was tested. Analytical monitoring of the test solutions was not performed.

11.01.2005 (28)

**Type** : Static
**Species** : Acartia tonsa
**Exposure period** : 48 hour(s)
**Unit** :
**Analytical monitoring** : No
**Method** : MAFF/U.K.OCNS/PARCOM
**Year** : 1994
**GLP** : Yes
**Test substance** : Grease with Calcium 12-hydroxystearate (CAS No. 3159-62-4) thickener and performance additives

**Result** : 48-h $EL_{50}$ >1000 mg/l WAF.
Immobilization in the 1000 mg/l WAF of the grease was 20% after 48 h. Control immobilization was 3%. Exposure of A. tonsa to the 1.0 mg/L 3,5-DCP solution resulted in 34% (32 organisms tested) immobilization. Numbers of immobilized A. tonsa were 1, 6, and 11 in the control, 1000 mg/l WAF, and 3,5-DCP reference, respectively.
The temperature range recorded during the test was 0.2 °C outside the recommended levels of 18 to 22 °C. This deviation was not considered significant.

**Test condition** : A 1000 mg/l water accommodated fraction was prepared by stirring 2 g of the grease in 2 L of artificial seawater for 24 h. The solution was allowed to stand for at least 1 h before the aqueous phase was drawn off and used undiluted as the test medium. Artificial seawater was prepared by dissolving artificial seasalts (Tropic Marin, Aquatechnik, Wartenberg, W. Germany) in reverse osmosis purified water to a salinity of 34 ± 2o/oo. Triplicate groups with 10 A. tonsa were exposed to 100 ml of test solution held in 200 ml glass crystallizing dishes. Three dishes containing artificial seawater only served as controls. Three dishes containing 1 mg/l solution of a reference compound 3,5-dichlorophenol (DCP) were also prepared. Aged A. tonsa (23 days old) from laboratory cultures were used. Original stocks were supplied by the Vandkvalitetsinstituttet, Copenhagen, Denmark.
Immobilized A. tonsa were recorded and removed from test vessels after 24 and 48 h. At the end of the test a few drops of formalin were added to the test vessels to preserve the organisms for subsequent counting. Test

was carried out in temperature controlled room set at 20 ± 2 °C with a 16h light, 8h dark illumination cycle. Test solutions were not renewed or aerated during test. Salinity ranged from 34 to 35o/oo. pH ranged from 8.2 to 8.4. Dissolved oxygen was 7.2 to 7.6 at 0 h and 6.8 to 7.0 at 48 h. Temperature throughout the test was 17.8 to 19.3 °C.

**Reliability** : (2) valid with restrictions
Only one concentration of the grease was tested. Analytical monitoring of the test solutions was not performed.

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### 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

**Species** : Skeletonema costatum (Algae)
**Exposure period** : 72 hour(s)
**Analytical monitoring** : No
**Method** : MAFF/U.K.OCNS/PARCOM, ISO
**Year** : 1994
**GLP** : Yes
**Test substance** : Grease with Calcium 12-hydroxystearate (CAS No. 3159-62-4) thickener and performance additives

**Method** : Williams test for NOELs
**Result** : In the limit test, the 1000 mg/l WAF of the grease produced significant adverse effects (>90%) on both average specific growth rate and area under the growth curve compared to the control over 72 h.
In the definitive test, the 72-h $EbL_{50}$ was between 100 and 1000 mg/l WAFs (close to 320 mg/l which resulted in 42% reduction).
The 72-h $ErL_{50}$ was between 320 and 1000 mg/l WAFs.
72-h NOEbL = 32 mg/l WAF.
72-h NOErL = 320 mg/l WAF.

| Nominal Conc. (mg/l) | 0-72 h % reduction (area) | 0-72h % reduction (growth rate) | 72 h mean chain conc (million chains/ml) |
|---|---|---|---|
| Control | -- | -- | 0.3 |
| 32 | 5 | -1.1 | 0.3 |
| 100 | 16 | 0.31 | 0.26 |
| 320 | 42 | 1.8 | 0.22 |
| 1000 | 100 | 100 | 0.007 |
| 3,5-DCP | 22 | -1.8 | 0.3 |

In the definitive test, the mean chain length in the starter culture was 2.6 cells/chain. At test termination, the mean chain lengths were 5.3, 5.3, 4.8, and 4.6 in the control, 32, 100, and 320 mg/l WAFs. No chains were visible in the 1000 mg/l WAF. Mean chain length in a 3,5-DCP flask was 3.7.

The reference compound 3,5-DCP produced reductions in growth rate and area under the growth curve that met the recommended MAFF criteria at 48 h. However, after 72 h only the area under the growth curve met the recommended criteria, since no inhibitory effects on growth rate relative to the control were observed. These results were not considered to invalidate the test as the response criteria was under regulatory review.

The pH change after 72 h in one of the control flask in the definitive test was 1.1 units which exceeded the increase of 1 unit recommended by ISO,

but it was not considered to have affected the integrity of the study.

**Test condition** : Two growth inhibition tests were performed. In the initial limit test, the 1000 mg/l WAF was prepared by stirring 2 g of the grease in 2 L of algal media for 24 h. The solution was allowed to stand for at least 1 h before the aqueous phase was drawn off and used undiluted as the test medium. Stock algal media was prepared by adding Analar grade salts in reverse osmosis water. The stock media were added to filtered, autoclaved seawater collected from an oyster hatchery at Reculver on the North Kent coast, UK, to prepare the culture and testing media following ISO recommendations (ISO/TC 147/SC5/WG 5 N/20, 1988). The second and definitive test consisted of WAFs prepared at loading rates of 32, 100, 320, and 1000 mg/l. Test vessels were 250 or 300 ml Erlenmeyer flasks containing 100 ml of test solution, inoculated with S. costatum to give an initial nominal cell concentration of 10,000 cells/ml. Three flasks were prepared for each loading rate and six flasks containing algal media only served as controls. Three flasks containing 100 ml of a 1.5 mg/l solution of 3,5-dichlorophenol (DCP) were also prepared. One uninoculated flask of each loading rate and control served as blanks. Flasks were covered with aluminum foil caps and incubated in a cooled, orbital incubator (100 cycles/min) under constant illumination (~3000 lux).
Chain/particle counts were made from all of the flasks at the start of the test and at 24-h intervals using a Coulter Counter. At test termination, mean chain length of S. costatum was estimated from a subsample from a control flask and one flask from each loading rate by microscopic examination in a Sedgewick Rafter cell. Effects of algal growth were evaluated by comparisons of areas under the growth curve and comparisons of average specific growth rates of the treatments relative to control. S. costatum used in the studies were laboratory cultures derived from a culture obtained from the culture collection maintained at the Scottish Marine Biological Association Laboratory in Oban, Scotland. Temperature ranged from 19.7 to 21.1 °C. pH ranged from 7.8 to 8.4 at 0 h and 8.6 to 9.1 at 72 h.

**Reliability** : (1) valid without restriction
11.01.2005 (28)

**Species** : Skeletonema costatum (Algae)
**Exposure period** : 72 hour(s)
**Analytical monitoring** : No
**Method** : MAFF/U.K.OCNS/PARCOM, ISO
**Year** : 1994
**GLP** : Yes
**Test substance** : Grease with Calcium 12-hydroxystearate (CAS No. 3159-62-4) thickener and performance additives

**Method** : Williams test for NOELs
**Result** : In the initial test and limit test, the 72-h $EbL_{50}$ > 1000 mg/l WAF.
The 72-h $ErL_{50}$ > 1000 mg/l WAF.
72-h NOEbL = 1000 mg/l WAF.
72-h NOErL = 1000 mg/l WAF.

**INITIAL TEST**:

| Nominal Conc. (mg/l) | 0-72 h % reduction (area) | 0-72h % reduction (growth rate) | 72 h mean chain conc (million chains/ml) |
|---|---|---|---|
| Control | ---- | ---- | 0.22 |
| 10 | -40 | -0.36 | 0.3 |

| | | | |
|---|---|---|---|
| 32 | -51 | -6.1 | 0.3 |
| 100 | -30 | -3 | 0.28 |
| 320 | -19 | -3.9 | 0.26 |
| 1000 | -18 | -12 | 0.25 |
| 3,5-DCP | 35 | 16 | 0.13 |

**LIMIT TEST**:

| **Nominal Conc. (mg/l)** | **0-72 h % reduction (area)** | **0-72h % reduction (growth rate)** | **72 h mean chain conc (million chains/ml)** |
|---|---|---|---|
| Control | ---- | ---- | 0.24 |
| 1000 | 27 | 8.4 | 0.19 |

**FINAL DCP TEST**:

| **Nominal Conc. (mg/l)** | **0-72 h % reduction (area)** | **0-72h % reduction (growth rate)** | **72 h mean chain conc (million chains/ml)** |
|---|---|---|---|
| Control | ---- | ---- | 0.3 |
| 3,5-DCP | 22 | -1.8 | 0.3 |

In the initial test, the mean chain length in the starter culture was 3.3 cells/chain. At test termination, the mean chain lengths were 4.4 and 4.7 in the control and 1000 mg/l WAF. Mean chain length in the starter culture was 3.2 cells/chain in the limit test and 5.3 and 4.8 in the control and 1000 mg/l WAF at the end of the test. Mean chain length in the starter culture was 2.6 cells/chain in the final DCP test and 5.3 and 3.7 in the control and 1.5 mg/l DCP at the end of the test.

The effects of the reference compound 3,5-DCP on average specific growth rate were outside the recommended criteria of 20 to 80% reduction. These results were not considered to affect the validity of the test as the response criteria was under regulatory review.

Temperature range in the initial test was outside the recommended limits although there were no adverse effects on control growth. The pH change after 72 h in the control in the final DCP test was 1.1 units which exceeded the increase of 1 unit recommended by ISO, but it was not considered to have affected the integrity of the study.

**Test condition** **:** Two growth inhibition tests were performed. In the initial test, WAFs with loading rates of 10, 32, 100, 320, and 1000 mg/l were prepared by adding the appropriate weight of grease to 2L of algal media and stirring for 24 h. The solutions were allowed to stand for at least 1 h before the aqueous phase was drawn off and used undiluted as test media. Stock algal media was prepared by adding Analar grade salts in Millipore Milli-Q filtered water. The stock media were added to filtered, autoclaved seawater collected from an oyster hatchery at Reculver on the North Kent coast, UK, to prepare the culture and testing media following ISO recommendations (ISO/TC 147/SC5/WG 5 N/20, 1988). Test vessels were 250 ml Erlenmeyer flasks containing 100 ml of test solution, inoculated with S. costatum to give an initial nominal cell concentration of 10,000 cells/ml. Three flasks were prepared for each loading rate and six flasks containing algal media only served as controls. Three flasks containing 100 ml of a 1.5 mg/l solution of 3,5-dichlorophenol (DCP) were also prepared. One uninoculated flask of each loading rate and control served as blanks. Flasks were covered with aluminum foil caps and incubated in a cooled,

orbital incubator (100 cycles/min) under constant illumination (~3000 lux). Chain/particle counts were made from all of the flasks at the start of the test and at 24-h intervals using a Coulter Counter. At test termination, mean chain length of S. costatum was estimated from a subsample from a control flask and one flask from the 1000 mg/l WAF by microscopic examination in a Sedgewick Rafter cell. Owing to concerns regarding the validity of the first test because of an exceedance in test temperature, a second limit test consisted of control, 1000 mg/l WAF, and 1 mg/l 3,5-DCP was conducted. The 3,5-DCP test was subsequently repeated at the correct concentration of 1.5 mg/l. Effects of algal growth were evaluated by comparisons of areas under the growth curve and comparisons of average specific growth rates of the treatments relative to control.
S. costatum used in the studies were laboratory cultures derived from a culture obtained from the culture collection maintained at the Scottish Marine Biological Association Laboratory in Oban, Scotland. Temperatures ranged from 19.8 to 23.9 °C, 20.0 to 20.4 °C, and 19.7 to 21.1 °C in the initial test, limit test, and the final 3,5-DCP test . In the initial test, pH was 8.1 at 0 h and 8.3 to 8.5 at 72 h. pH ranged from 8.2 to 8.3 at 0 h and 8.9 to 9.0 at 72 h in the limit test. In the final DCP test, control pH was 7.8 at 0 h and 8.9 at 72 h and DCP solution pH was 8.4 at 0 h and 9.0 at 72 h.

**Reliability** : (1) valid without restriction
11.01.2005 (27)

### 4.9 ADDITIONAL REMARKS

**Memo** : Aquatic toxicity of dissociation products of grease thickeners

**Remark** : The physical consistency of grease thickeners (Section 1.1.1), the manner in which they are produced (Section 1.1.1), and their low solubility all contribute to a low risk of exposure to aquatic organisms (Sections 2.14 and 2.6.1). When aquatic organisms were tested against whole grease thickened with calcium soap, calcium 12-hydroxystearate, or mixed tallow and calcium 12-hydroxystearate, either no toxicity was observed, or effects were in the 100 to 1000 mg/l range (see Sections 4.1, 4.2 and 4.3). These values can also be used as read-across data for lithium salts. As shown below for ECOSAR estimates of aquatic toxicity, lithium salts of fatty acids C9 (nonanedioc dilithium salt) and C22 (docosanoic lithium salt) would be expected to show no toxicity at the limit of these compound's water solubility. 12-hydroxy-octadecanoic lithium salt is expected to show only slight toxicity.

The low hazard of these products extends to their dissociation products, as noted below for various components of grease thickeners and surrogate structures. Although it is not likely that aquatic exposure to dissociation products of grease thickeners will occur, if such instances arise, the toxicity of the fatty acid moiety is expected to be low.
Toxicity to the lithium ion, should that occur, is shown to be in the 6 to >100 mg/l range.

| Substance<br>Test<br>Animal | Endpoint | Value mg/l | Source |
|---|---|---|---|
| Fatty Acids and salts | | | |
| C9 nonanedioic acid, dilithium salt | | | |
| [water solubility 1000 mg/l (WSKOWWIN V1.41) | | | |
| Fish | LC/$EC_{50}$ | (a) | US EPA 2000 |
| Invertebrate | | | ECOSAR V0.99 |
| Algae | | | |
| C16 palmitic, Na salt | | | |
| [water solubility 33 mg/l (WSKOWWIN V1.41) | | | |
| Goldfish | Lethal dose | 11 | P & G, 2003 |
| Red killifish | 96-h $LD_{50}$ | 150 | |
| Invertebrates | Lethal conc. | (a) | |
| C18 stearic, Na salt | | | |
| [water solubility 3.3 mg/l (WSKOWWIN V1.41) | | | |
| Goldfish | Lethal dose | 14 | P & G, 2003 |
| Red killifish | 96-h $LD_{50}$ | 125 | |
| Invertebrates Lethal conc. | | (a) | |
| Algae | $EC_{50}$ | >1016 | |
| | NOEC | 1016 | |
| C18 stearic, Ca salt | | | |
| [water solubility <0.1 µg/l (WSKOWWIN V1.41) | | | |
| Fish | $LC_{50}$ | (a) | US EPA 2000 |
| | | | ECOSAR V0.99 |
| C18 octadecanoic acid, 12 hydroxy- Li salt | | | |
| [water solubility 222 mg/l (WSKOWWIN V1.41) | | | |
| Fish | $LC_{50}$ | 123 | US EPA 2000 |
| | | | ECOSAR V0.99 |
| C22 docosanoic acid | | | |
| [water solubility 0.016 mg/l (SIDS 2001) | | | |
| Zebrafish | 96-h $LC_{50}$ | >5 | SIDS, 2001 |
| | 14-d $LC_{50}$ | >5 | |
| Invertebrates | 48-h $EC_{50}$ | >5 | |
| | 21-d $EC_{50}$ (repro) | >0.84 | |
| | 21-d NOEC (repro) | >0.84 | |
| C22 docosanoic acid, Li salt | | | |
| [water solubility 0.04 mg/l (WSKOWWIN V1.41) | | | |
| Fish | | | |
| Invertebrates | LC/$EC_{50}$ | (a) | US EPA 2000 |
| Algae | | | ECOSAR V0.99 |
| METAL | | | |

| | | | | |
|---|---|---|---|---|
| | Lithium chloride | | | |
| | Fish | 96-h $LC_{50}$ | 315 | US EPA 2004 |
| | | | 22 | |
| | | | 62 | |
| | | | 65 | |
| | | | >105 | |
| | | | 186 | |
| | Lithium sulfate | | | |
| | Invertebrates | 24-h $LC_{50}$ | 39.3 | |
| | | | 6.48 | |
| | | 24-h $EC_{50}$ | 44.8 | |
| | | 96-h $EC_{50}$ | 9.3 | |
| | Algae | 3-4 month | | |
| | | LOEC | 160 | |
| | | NOEC | 80 | |
| | (a) Not acutely toxic at limits of solubility | | | |
| 11.01.2005 | | | | (25) (26) (32) (33) |

## 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 5000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | Male/female |
| **Number of animals** | : | 10 |
| **Vehicle** | : | Undiluted |
| **Doses** | : | 5000 mg/kg only |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Lithium complex Grease |
| **Method** | : | Five male and five female fasted rats were given a single oral dose (5000 mg/k) of the test material. The rats were observed 1, 4 and 24 hours after administration of the test material for clinical signs of toxicity and any other pharmacological signs. Body weights were recorded before administration of the test material and again on days 7 and 14.<br>All animals were sacrificed on day 14 and a gross necropsy was performed on each of them. Abnormal observations were recorded. |
| **Result** | : | No clinical signs were observed and no animal died during the study. There was a body weight increase for all animals on the study. At necropsy there were no abnormal observations.<br>The $LD_{50}$ of the test material was greater than 5000 mg/kg. |
| **Test substance** | : | The grease had the following composition<br><br>Wt % base oil ~65<br><br>Thickeners<br>Li 12-hydroxy stearate 13.1%<br>Dilithium azelate 2.6%<br><br>Wt % other additives ~20 |
| **Reliability** | : | (1) valid without restriction |

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| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 10000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Albino |
| **Vehicle** | : | Corn oil |
| **Doses** | : | 0.05-10.0 g/kg |
| **Year** | : | 1982 |
| **GLP** | : | No data |
| **Test substance** | : | Magnesium stearate |
| **Result** | : | The publication states:<br>Given as 25% suspension in corn oil.<br><br>Animals fasted overnight and then given dose ranging from 0.05 to 10.0 g/kg. Animals observed daily for 14 days. All animals at 10.0 g/kg exhibited mild diarrhea. |
| **Reliability** | : | (4) not assignable<br>Information is taken from the report of a Cosmetic ingredient review panel. |

Original data not available.

09.12.2003 (4)

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | 5000 - 15000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Albino |
| **Vehicle** | : | Propylene glycol |
| **Doses** | : | 0.05, 1, 3 & 15 g/kg |
| **Year** | : | 1982 |
| **GLP** | : | No data |
| **Test substance** | : | Lithium stearate |

**Result** : Lithium stearate was administered in propylene glycol (concentration unspecified) to 30 albino rats (sex not specified).

The publication states:

Animals fasted for 24 hrs. and then given dosages ranging from 0.05 to 15.0 g/kg. Animals dosed at 0.05, 1.0 and 3.0 g/kg showed no toxic effects; all animals administered 15.0 g/kg died within 16 hrs. having exhibited unkempt coats, impaired locomotion and lethargy prior to death.

**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel.
Original data not available.

24.12.2003 (4)

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 3000 mg/kg bw |
| **Species** | : | Rabbit |
| **Strain** | : | New Zealand white |
| **Sex** | : | Male/female |
| **Number of animals** | : | 10 |
| **Vehicle** | : | Undiluted |
| **Doses** | : | 300 mg/kg |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Lithium complex Grease |

**Method** : Undiluted test material was applied to the shorn dorsal skin of five male and five female NZW rabbits. The applied grease was covered with an occlusive dressing which was left in place for 24 hours. Following the 24 hours exposure period the covering was removed and any residual test material was wiped from the skin using mineral oil and a gauze. Observations were recorded daily throughout the following 14 days. Body weights were recorded prior to application of the test material and again on days 7 and 14. All rabbits were killed by lethal injection and a gross necropsy was performed and a record made of any abnormalities.

**Result** : There were no clinical signs of toxicity during the study and no animals died. Erythema and edema was observed at the treated skin site when the occlusive covering was removed.
At this time average erythema and edema scores were 2.6 and 2 respectively (same average scores for each sex). The skin responses gradually subsided and by day 6 had completely disappeared. Animals

gained weight during the study and no abnormalities were observed at necropsy.
The dermal LD50 was therefore greater than 3000 mg/kg.

**Test substance** : The grease had the following composition

Wt % base oil ~65

Thickeners
Li 12-hydroxy stearate 13.1%
Dilithium azelate 2.6%

Wt % other additives ~20

**Reliability** : (1) valid without restriction
11.01.2005 (19)

### 5.2.1 SKIN IRRITATION

**Species** : Rabbit
**Concentration** : Undiluted
**Exposure** : Semiocclusive
**Exposure time** : 4 hour(s)
**Number of animals** : 6
**Vehicle** : None
**Year** : 1944
**GLP** : Yes
**Test substance** : Lithium complex Grease

**Method** : 0.5 ml of undiluted test material was applied to three separate sites on the shorn dorsal trunks of three male and three female NZW rabbits. Each site was covered with a semiocclusive dressing. One site was abraded, the other two were intact skin.
One of the intact skin sites was only covered for 4 hours and the other two sites were covered for 24 hours. At the end of the exposure periods, residual test material was removed from the skin using gauze and mineral oil.
After patch removal, the test site was examined for erythema and edema and the responses were scored immediately using the standard Draize scale. Skin responses were scored again at 1, 24, 48 and 72 hours after patch removal and again on days 4 through 6.
Body weights of animals were recorded before application of test material and again at the end of the study.

**Result** : No clinical signs of toxicity were observed and all animals gained weight over the course of the study.
Average scores for erythema and edema are as shown in the following table.

| **Time** | **4 hour** | | **24 hour exposure** | | | |
|---|---|---|---|---|---|---|
| | **Erythema** | **Edema** | **Erythema** | | **Edema** | |
| | | | **I*** | **A** | **I** | **A** |
| 0 hrs | 0.7 | 0 | 3.2 | 3.2 | 2.7 | 2.8 |
| 1 hr | 0.7 | 0 | 3.2 | 3.2 | 2.7 | 2.8 |
| 24 hrs | 0.2 | 0.2 | 3 | 3.2 | 2.3 | 2.3 |
| 48 hrs | 0.2 | 0.2 | 2 | 2.2 | 1.7 | 2 |
| 72 hrs | 0.2 | 0 | 1.5 | 1.7 | 1.3 | 1.5 |
| Day 4 | 0 | 0 | 1 | 1 | 0.5 | 0.7 |
| Day 5 | | | 0.2 | 0.2 | 0 | 0 |

| | | |
|---|---|---|
| | Day 6 | 0 0 0 0 |

* I = Intact, A = Abraded

The four hour exposures resulted in only slight irritation which had cleared by day 4.

24 hour exposure caused moderate to severe erythema with well defined to severe edema. Skin responses had cleared by day 6 and there was no evidence that abraded skin was more irritated than intact skin.

The calculated Primary irritation indices were:
4 hour exposure 0.38
24 hour exposure 4.92

**Test substance** : The grease had the following composition

Wt % base oil ~65

Thickeners
Li 12-hydroxy stearate 13.1%
Dilithium azelate 2.6%

Wt % other additives ~20

**Reliability** : (1) valid without restriction
11.01.2005 (21)

**Species** : Rabbit
**Concentration** : Undiluted
**Exposure time** : 4 hour(s)
**Number of animals** : 6
**Vehicle** : None
**PDII** : 0
**Result** : Not irritating
**Year** : 1982
**GLP** : No data
**Test substance** : Magnesium stearate

**Method** : Two studies were summarized:
A four hour study of acute dermal corrosion and a 24 hour study for skin irritation.
In both studies 6 albino rabbits were used.
The test material was applied under an occlusive dressing in both studies.
Also in both studies half the test sites were abraded while the other half were intact skin.

The corrosion study was conducted according to the procedure described in 49 CFR 173.240 (a) (1).

**Result** : The primary irritation index in both studies was 0.
**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel.
Original data not available.
09.12.2003 (4)

### 5.2.2 EYE IRRITATION

| | | |
|---|---|---|
| **Species** | : | Rabbit |
| **Concentration** | : | Undiluted |
| **Dose** | : | 0.1 ml |
| **Number of animals** | : | 6 |
| **Vehicle** | : | None |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Lithium complex Grease |

**Method** : 0.1 ml of test material was placed in the conjunctival sac of the right eye of six female NZW rabbits. The left eye was untreated and served as control. The eyes were examined at 1, 24, 48 and 72 hours after treatment and again on day 7. Ocular reactions were scored according to the standard Draize scale.
Body weights were recorded at the beginning and the end of the study.

**Result** : Conjunctival redness was observed in all animals 1 hour after application of the test material and in three animals at 24 hours. This conjunctival response continued in one animal for 72 hours but was not seen in any animal after 7 days.
Iritis was observed in only one animal at 24 hours and corneal opacity also occurred at 24 hours in the same animal and this persisted for 24 hours. All eyes were normal after 7 days.

The average Draize scores for 6 rabbits are shown in the following table.

| Time after application of test material | Cornea | Iris | Conjunctivae |
|---|---|---|---|
| 1 hour | 0 | 0 | 10 |
| 24 hours | 0.8 | 0.8 | 3.3 |
| 48 hours | 0.8 | 0 | 2.7 |
| 72 hours | 0 | 0 | 1.3 |
| 7 Days | 0 | 0 | 0 |

**Test substance** : The grease had the following composition

Wt % base oil ~65

Thickeners
Li 12-hydroxy stearate 13.1%
Dilithium azelate 2.6%

Wt % other additives ~20

**Reliability** : (1) valid without restriction
11.01.2005 (22)

| | | |
|---|---|---|
| **Species** | : | Rabbit |
| **Concentration** | : | Undiluted |
| **Comment** | : | Not rinsed |
| **Number of animals** | : | 6 |
| **Vehicle** | : | None |
| **Result** | : | Not irritating |
| **Year** | : | 1982 |
| **GLP** | : | No data |
| **Test substance** | : | Magnesium stearate |

| | | |
|---|---|---|
| **Result** | : | The scores were zero on days 1, 2 and 3 |
| **Reliability** | : | (4) not assignable<br>Information is taken from the report of a Cosmetic ingredient review panel.<br>Original data not available. |

09.12.2003 (4)

## 5.3 SENSITIZATION

| | | |
|---|---|---|
| **Type** | : | Buehler Test |
| **Species** | : | Guinea pig |
| **Concentration** | : | 1st: Induction undiluted occlusive epicutaneous<br>2nd: Challenge undiluted occlusive epicutaneous |
| **Number of animals** | : | 10 |
| **Vehicle** | : | None |
| **Result** | : | Not sensitizing |
| **Year** | : | 1997 |
| **GLP** | : | Yes |
| **Test substance** | : | Lithium complex grease |

**Method** : On the basis of the results of a preliminary irritation screen, it was decided to use undiluted test material for the induction and challenge dosing in the sensitization test.
The test material was applied under a Hilltop chamber to the shorn skin of 10 male and 10 female Guinea pigs. The patches were allowed to remain in place for six hours, after which they were removed and any residual test material was also removed from the skin using a gauze and mineral oil.
The treated sites were examine after each dosing day and scored for dermal irritation at 24 and 48 hours. This dosing and scoring procedure was performed once a week for three weeks.
A concurrent positive control group of five animals (3 males and 2 females) was treated with 0.3% 1-chloro-2,4-dinitrobenzene in 80% ethanol (ethanol in distilled water).
An additional group of ten animals (5 of each sex) was treated with vehicle (mineral oil).

Fourteen days after the last induction dose, the animals were challenged by applying material in the same manner as the induction applications but on a naive site.
The vehicle control group was challenged with mineral oil and test substance.
The positive control group animals were challenged with DNCB at 0.01% and 0.2% in acetone.
All animals were observed for local and systemic effects.
24 hours after challenge, the animals were depilated. After a minimum of 2 hours following depilation the test sites were assessed and graded (24

hour grade) and were graded again after a further 24 hours (48 hour grade).

When skin reactions were graded throughout the study scores were attributed to each test site on a scale of 0-3 for erythema.
After the sensitization doses a score of 1 or more was taken to indicate that sensitization had occurred. Furthermore if the test reactions exceeded the most severe control reactions, the animal was considered to be sensitized.

**Result** : A summary of the challenge scores is given in the following table.

| Test Group | % animals with score at 24 hours 0 | + | 1 | 2 | 3 |
|---|---|---|---|---|---|
| Vehicle control Induced with mineral oil | | | | | |
| Mineral oil challenge | 100 | 0 | 0 | 0 | 0 |
| Test material challenge | 100 | 0 | 0 | 0 | 0 |
| Test material induced with neat test material | | | | | |
| Test material challenge | 100 | 0 | 0 | 0 | 0 |
| Positive control animals induced with 0.3% DNCB | | | | | |
| 0.01% DNCB challenge | 60 | 20 | 20 | 0 | 0 |
| 0.2% DNCB challenge | 0 | 0 | 20 | 0 | 80 |

The positive control data clearly demonstrate the sensitivity of the test method. The test material itself did not cause skin sensitization in this study.

**Test substance** : The grease had the following composition

| | |
|---|---|
| Wt % base oil | ~80 |
| Thickeners | |
| Li 12-hydroxy stearate | 8.8% |
| Dilithium azelate | 1.8% |
| Wt % other additives | ~10 |

**Reliability** : (1) valid without restriction
11.01.2005 (23)

## 5.4 REPEATED DOSE TOXICITY

**Type** : Sub-chronic
**Species** : Rat
**Sex** : Male/female
**Strain** : Wistar
**Route of admin.** : Oral feed
**Exposure period** : 3 Months
**Frequency of treatm.** : Daily in the diet
**Doses** : 5, 10 & 20% in the diet
**Control group** : Yes
**NOAEL** : 5 %
**Year** : 1980
**GLP** : No data
**Test substance** : Magnesium stearate

**Method** : Groups of 20 male and 20 female six week old rats were fed diets containing 5, 10 or 20 magnesium stearate. The diets were semi synthetic in which sodium caseinate replaced casein. The carbohydrates of the diet

were substituted by magnesium stearate as follows:

| Group | Magnesium stearate % in diet | Carbohydrate % in diet |
|---|---|---|
| Contro l | 0 | 67.3 |
| | 5 | 62.3 |
| | 10 | 57.3 |
| | 20 | 47.8 |

The diets fed were considered isocaloric, as stearate has a calorific value of about 9, and a pilot study demonstrated that 35-40% of the stearate is absorbed at a 10% level in the diet. Acidified water (pH 3.5) was available ad libitum.
The animals were weighed once weekly and food utilization and weight gain was calculated for each sex of all groups of rats.
Blood samples were taken from 8 males and 8 females from each group prior to dosing and at 8 and 12 weeks. The following hematological and clinical chemistry determinations were made:

Hematology
Hemoglobin
packed cell volume (PCV)
red cell count
total white cell count
reticulocyte count
differential white cell count.

Clinical chemistry
Glucose
urea
aspartate amino transferase
alkaline phosphatase

At the termination of the study, the rats were sacrificed and the following organs were weighed: thymus, liver, kidneys, adrenals, testes/ovaries, heart, lungs, brain and pituitary.
Samples of the organs listed above and the following tissues were taken for light microscopy: urinary bladder, stomach, duodenum, pancreas, jejunum, cecum, colon, thyroid, parathyroid, triceps, brachial muscle, ischiadic nerve, axillar lymph node, uterus, sternum, eye, Harderian gland, skin and submandibular gland. Microscopic examination was undertaken on the high dose and control animals only.

**Result** **:** The weight gains of the 20% males were significantly less than he corresponding controls during the first 8 weeks of the study [No actual data given in the publication].
Concomitantly these animals were quiet with slow and unsteady movements. Four males in his group died within the first 2 months and all had stone formation in the lower urinary pathways and the deaths were considered to be related to this finding. One other male in this group was incontinent. In the remaining males, the symptoms receded during the following 4 weeks. There were no clinical effects in females in any group.
A reduction in PCV [P<0.01, but no data provided] was found in the 20% males compared to controls. No other hematological differences were reported.

In addition to the findings reported in the males that died in the 20% group, changes were also found in the renal pelvis and in the lower urinary pathways (due to stone formation) at autopsy in 4 males and one female in the 20 % group.

The relative liver and kidney weights recorded were as follows:

| Dietary concentration | Sex | Liver g/100g body wt ±SD | Kidney g/100g body wt. ±SD |
|---|---|---|---|
| 0 | M | 3.25±0.21 | 633±48.6 |
| 5 | M | 3.13±0.21* | 614±51.5 |
| 10 | M | 2.99±0.23*** | 599±40.6* |
| 20 | M | 2.82±0.18*** | 640±80.7 |
| 0 | F | 3.30±0.24 | 768±103 |
| 5 | F | 3.33±0.18 | 661±86.5*** |
| 10 | F | 3.31±0.31 | 667±54.0*** |
| 20 | F | 3.16±0.23* | 646±55.8*** |

* P< 0.05
*** P<0.001

Nephrocalcinosis was seen in all females and in 12/20 males in the control group. In 18 of the females nephrocalcinosis was regarded as severe. Slight to moderate nephrocalcinosis was observed in 19/20 of the females in the 20% group and 7/20 of the males were affected only slightly. Deposition of iron was found in various amounts in kidney and in liver, the amount was increased in the liver of both sexes in the 20% group. Liver glycogen showed a marked decrease in males in the 20% group and no difference was found in the females.

The authors comment that :

the occurrence of nephrocalcinosis is a common finding in animals fed semi-synthetic diets. The increased magnesium content of the diet could explain the reduction of nephrocalcinosis in the 20% animals.
A high magnesium content of the diet has also been previously associated with a greater incidence of stone formation in the lower part of the urinary tract.

The authors concluded that:

when liver weight was used as a measure of adverse effect, the no effect level was estimated to be 5% magnesium stearate in the diet, corresponding to 2500 mg/kg body weight.

**Reliability** : (2) valid with restrictions
Few experimental details are provided and detailed results are not included in the publication.
However, the publication does provide useful information on the effects of repeated oral exposure to magnesium stearate.

24.12.2003 (29)

**Type** : Sub-chronic
**Species** : Rat
**Sex** : Male/female
**Strain** : Sprague-Dawley
**Route of admin.** : Gavage
**Exposure period** : 90 days
**Frequency of treatm.** : Daily, seven days each week
**Doses** : 250, 500 & 1000 mg/kg/day

| | | |
|---|---|---|
| **Control group** | : | Yes, concurrent vehicle |
| **NOAEL** | : | 1000 mg/kg |
| **Year** | : | 1977 |
| **GLP** | : | Yes |
| **Test substance** | : | R960002575 |

**Method** :

Sprague-Dawley rats were used in this study. The animals (males and females) were aged 6 weeks at the beginning of the study.
The test material was administered orally by gavage at doses of 250, 500 or 1000 mg/kg/day in a dose volume of 4 ml/kg to groups of ten male and ten females for each dose level.
Additionally, a group of ten male and ten females served as vehicle controls and for these corn oil alone (4ml/kg) was administered. This treatment was continued daily, seven days each week for 90 days.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses.
Body weights and food intakes were recorded weekly.
At the end of the study, on day 91, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

<u>Hematology</u>
Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Reticulocyte count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts
Erythrocyte morphology
Reticulocyte count

<u>Clinical chemistry</u>
Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen
Fasting glucose
Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Total bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus
Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Aorta
Bone (sternum/femur with articular surface)
Brain (medulla/pons, cerebrum and cerebellum)
Epididymis (2)
Esophagus
Eye with optic nerve*
Heart
Kidneys (2)
Large intestine (cecum, colon and rectum)
Lacrimal gland*
Liver (2 sections)
Lung with mainstem bronchi
Lymph node (mediastinal)
Lymph node (mesenteric)
Mammary gland*
Muscle (biceps femoris)*
Nasal turbinates
Nerve (sciatic)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland (submaxillary)
Seminal vesicles
Skin (treated and untreated)
Small intestine ( duodenum, ileum and jejunum)
Spinal cord (cervical, thoracic, lumbar)*
Spleen
Stomach
Testes
Thymic region
Thyroid (with parathyroids)
Trachea
Urinary bladder
Uterus (body/horns with cervix)
Zymbal's gland*
Macroscopic lesions
Target organs
All the above tissues from all the animals in the high dose group and the controls were examined microscopically, except those indicated * which were preserved but not examined.

Statistical analysis
Body weight, body weight change from week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used.
In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** : There were no mortalities during the study and there were no treatment-related clinical signs of toxicity. There were no adverse effects of treatment observed during the ophthalmoscopic examinations. Body weights were unaffected by treatment. The food consumption values for the 500 and 1000 mg/kg groups were often higher than the controls. However, they were considered to be within normal ranges and not treatment-related.

All except the following hematological parameters were unaffected by treatment. Those listed below were within the normal range for the laboratory and were not considered to be of toxicological significance.
Prothrombin time increases in males only:
15% in 500 mg/kg/day group
19% in 1000 mg/kg/day group
Activated partial thromboplastin time increase
18% in 250 and 1000 mg/kg/day groups

The only difference in clinical chemistry was a 9% increase in the phosphate levels of the 500 mg/kg/day females. This difference was not considered to be a treatment-related effect.

There were no effects on either organ weights, organ/body weight ratios or organ/brain weight ratios.

There were no macroscopic findings at necropsy and no treatment-related microscopic findings.

The NOAEL was considered to be 1000 mg/kg/day.

**Test substance** : R960002575 is a Lithium complex grease with the following composition
Wt % base oil ~80

Thickeners

Li 12-hydroxy stearate 8.8%
Dilithium azelate 1.8%

Wt % other additives ~10

The test material was prepared as solutions in corn oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 4 |
| II | 250 | 62.5 | 4 |
| III | 500 | 125 | 4 |
| IV | 1000 | 250 | 4 |

**Reliability** : (1) valid without restriction
03.12.2004 (10)

**Type** : Sub-acute
**Species** : Rat
**Sex** : Male/female
**Strain** : Sprague-Dawley
**Route of admin.** : Dermal
**Exposure period** : Six hours daily
**Frequency of treatm.** : Daily, five days each week for four weeks
**Post exposure period** :
**Doses** : 525, 1050 & 2100 mg/kg/day
**Control group** : Yes, concurrent vehicle
**NOAEL** : 2100 mg/kg bw
**Method** :
**Year** : 1977
**GLP** : Yes
**Test substance** : R960002575

**Method** : Male and female Sprague-Dawley rats aged approximately 7 and 9 weeks respectively were used in this study.
The test material was applied to the shorn skin of groups of five male and five females for each dose level.
Additionally, a group of five male and five females served as vehicle controls and for these mineral oil alone was applied. The test sites were covered with an occlusive dressing which was left in place for six hours. After this time, the dressings were removed and any residual test material was removed from the skin using a gauze and mineral oil. This treatment was continued daily, five days each week for four weeks.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses.
Body weights and food intakes were recorded weekly.
At the end of the study, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

Hematology
Hemoglobin concentration
Hematocrit
Erythrocyte count

Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts
Erythrocyte morphology
Reticulocyte count

Clinical chemistry
Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen
Fasting glucose
Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Total bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus
Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Brain (medulla/pons, cerebrum and cerebellum)
Heart
Kidneys (2)
Liver (2 sections)
Ovaries (2)
Skin (treated and untreated)
Spleen
Testes with epididymides (2)

All the above tissues from all the animals in the high dose group and the controls were examined microscopically.

Statistical analysis
Body weight, body weight change from week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were

analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used.
In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** : All animals survived throughout the study and there were no clinical signs of toxicity and no dermal irritation was observed in the treatment groups.
Body weights were unaffected by treatment except that at four weeks the 2100 mg/kg/day males weighed approximately 3% less than the corresponding controls. However, this difference was not statistically significant.
Food consumption of the treatment groups were generally similar to the controls. A slight increase in food consumption of the mid dose males and high dose females at weeks one and two respectively were not considered to be of biological relevance.
Hematological and clinical chemical parameters, organ weights and microscopic findings were all unaffected by treatment.
It was concluded that the NOAEL was 2100 mg/kg/day.

**Test substance** : R960002575 is a Lithium complex grease with the following composition

Wt % base oil ~80

Thickeners
Li 12-hydroxy stearate 8.8%
Dilithium azelate 1.8%

Wt % other additives ~10

The test material was prepared as solutions in mineral oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 2.1 |
| II | 525 | 250 | 2.1 |
| III | 1050 | 500 | 2.1 |
| IV | 2100 | 1000 | 2.1 |

**Reliability** : (1) valid without restriction
03.12.2004 (11)

| | | |
|---|---|---|
| **Type** | : | Sub-chronic |
| **Species** | : | Rat |
| **Sex** | : | Male/female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Dermal |
| **Exposure period** | : | Six hours daily |
| **Frequency of treatm.** | : | Daily, five days each week for 13 weeks |
| **Post exposure period** | : | |
| **Doses** | : | 525, 1050 & 2100 mg/kg/day |
| **Control group** | : | Yes, concurrent vehicle |
| **NOAEL** | : | 2100 mg/kg |
| **Year** | : | 1997 |
| **GLP** | : | Yes |
| **Test substance** | : | R960002575 |

**Method** :

Male and female Sprague-Dawley rats aged 7 and 9 weeks respectively were used in this study.
The test material was applied to the shorn skin of groups of ten male and ten females at doses of 525, 1050 or 2100 mg/kg/day. Additionally, a group of ten male and ten females served as vehicle controls and for these animals mineral oil alone was applied. The test sites were covered with an occlusive dressing which was left in place for six hours. After this time, the dressings were removed and any residual test material was removed from the skin using a gauze and mineral oil. This treatment was continued daily, five days each week for 13 weeks.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses. Examination of the skin for irritation was undertaken pre-test and then daily during the first week of exposure and weekly thereafter.
Body weights and food intakes were recorded weekly.
At the end of the study, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

<u>Hematology</u>
Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts
Erythrocyte morphology
Reticulocyte count

<u>Clinical chemistry</u>
Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen
Fasting glucose

Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Total bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus
Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Aorta
Bone (sternum/femur with articular surface)
Brain (medulla/pons, cerebrum and cerebellum)
Epididymis (2)
Esophagus
Eye with optic nerve*
Heart
Kidneys (2)
Large intestine (cecum, colon and rectum)
Lacrimal gland*
Liver (2 sections)
Lung with mainstem bronchi
Lymph node (mediastinal)
Lymph node (mesenteric)
Mammary gland*
Muscle (biceps femoris)*
Nasal turbinates
Nerve (sciatic)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland (submaxillary)
Seminal vesicles
Skin (treated and untreated)
Small intestine ( duodenum, ileum and jejunum)
Spinal cord (cervical, thoracic, lumbar)*
Spleen
Stomach
Testes
Thymic region
Thyroid (with parathyroids)

Trachea
Urinary bladder
Uterus (body/horns with cervix)
Zymbal's gland*
Macroscopic lesions
Target organs
All the above tissues from all the animals in the high dose group and the controls were examined microscopically, except those indicated * which were preserved but not examined.

Statistical analysis
Body weight, body weight change from week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used.
In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** **:** There were no treatment-related deaths and there were no clinical signs of toxicity throughout the study. Although mild skin irritation was seen sporadically, it was not regarded as treatment-related. There were no treatment-related changes seen in the ophthalmoscopic examinations.
Apart from the mid dose males there were no treatment-related effects on body weight. In the case of the mid dose males, they were slightly lower than the controls throughout, but since animals in the higher dose group were unaffected this finding is not considered toxicologically significant.
Food consumption was unaffected by exposure to test material.
There were no biologically significant effects on either the hematology or clinical chemistry determinations that were undertaken.
Terminal organ weights, organ/body weight ratios and organ/brain weight ratios were unaffected by treatment.

There were no treatment-related macroscopic observations at necropsy and after histology, no microscopic changes were observed that were considered to be treatment-related.

**Test substance** **:** R960002575 is a Lithium complex grease with the following composition

Wt % base oil ~80

Thickeners
Li 12-hydroxy stearate 8.8%
Dilithium azelate 1.8%

Wt % other additives ~10

The test material was prepared as solutions in mineral oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 2.1 |
| II | 525 | 250 | 2.1 |
| III | 1050 | 500 | 2.1 |
| IV | 2100 | 1000 | 2.1 |

**Reliability** : (1) valid without restriction
03.12.2004 (12)

**Type** : Sub-chronic
**Species** : Rat and Mouse
**Sex** : Male/female
**Strain** : Rat F344; Mouse B6C3F1
**Route of admin.** : Oral feed
**Exposure period** : 90 days
**Frequency of treatm.** : Continual in the diet
**Doses** : 0.62, 1.25, 2.5, 5 & 10 % in the diet
**Control group** : Yes
**Year** : 1992
**GLP** : Yes
**Test substance** : Castor oil

**Method** : 10 animals of each sex and of each species were used for each dose group.
The treatment groups were fed diets containing either 0.62, 1.25, 2.5, 5 or 10 % castor oil. In addition an extra 10 rats of each sex for each dietary level were fed for 21 days and these animals were used to provide blood samples for hematological and clinical chemical determinations on days 5 and 21, after which they were killed.
The main study animals were observed regularly throughout the study for clinical signs and were also weighed weekly.
Food consumption was also recorded throughout the study.
At the end of the study at 13 weeks, all animals underwent a complete necropsy. Blood samples were taken for the following hematological and clinical chemical measurements.

Hematology: Red blood cell count, examination of red blood cell morphology, hematocrit, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, differential white cell count, reticulocyte count (absolute) and platelet count (absolute).

Clinical chemistry: alkaline phosphatase, albumin, urea nitrogen, creatinine, alanine aminotransferase activity, total bile acids, sorbitol dehydrogenase activity, total protein and creatinine kinase activity.

The following organs were weighed: liver, right kidney, right testicle, heart,

thymus and lungs.

The following tissues were examined histopathologically in all control and high dose rats and mice: Adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testis or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur (including marrow), heart, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, rectum, salivary glands, skin, spinal cord and sciatic nerve (if neurological signs present), spleen, forestomach and glandular stomach, thymus, thyroid gland, trachea, urinary bladder, Zymbal glands, all gross lesions and tissue masses including lymph nodes.
In addition the livers from male rats of all other dose groups were examined.

Reproductive toxicity screen
Sperm motility and sperm density was assessed at necropsy.
Additionally for the 12 days prior to necropsy, females were subject to a vaginal lavage with saline. The aspirate was stained and examined to enable an assessment to be made of the stages of the estrous cycle.

Statistical analysis
Body weight and organ weight data were examined within each sex by one-way analysis of variance followed by Dunnett's t-test if pair-wise comparisons were indicated ($P<0.05$).

**Result** : The following is taken from the abstract of the report:

Exposure to castor oil at dietary concentrations as high as 10% in 13-week studies did not affect survival or body weight gains of rats or mice (10 per sex and dose). There were no biologically significant effects noted in hematologic analyses in rats. Mild increases in total bile acids and in serum alkaline phosphatase were noted at various times during the studies in rats receiving the higher dietary concentrations of castor oil. Liver weights were increased in male rats receiving the 10% dietary concentration and in male and female mice receiving diets containing 5% or 10% castor oil. However, there were no histopathologic lesions associated with these liver changes, nor were there any compound-related morphological changes in any organ in rats or mice. No significant changes were noted in a screening for male reproductive endpoints, including sperm count and motility, and no changes were observed in the length of estrous cycles of rats or mice given diets containing castor oil. Thus, no significant adverse effects of castor oil administration were noted in these studies.

**Test substance** : USP AA grade castor oil was used.
It was incorporated in the diet and checks were made of actual dietary concentrations. These were as follows:

| **Target concentration (%)** | **Actual concentration (%)** |
|---|---|
| 0.62 | 0.62 |
| 1.25 | 1.26 |
| 2.5 | 2.64 |
| 5 | 4.91 |
| 10 | 9.67 |

**Reliability** : (1) valid without restriction

24.12.2003 (18)

## 5.5 GENETIC TOXICITY 'IN VITRO'

| | | |
|---|---|---|
| **Type** | : | Ames test |
| **Result** | : | Negative |
| **Test substance** | : | Magnesium stearate |
| **Remark** | : | A cosmetic ingredients review panel concluded that magnesium stearate was not a mutagen in microbial tests with Salmonella typhimurium TA-1535, TA-1537, TA-1538 and Saccharomyces cerevisiae D4 with or without metabolic activation by liver and lung preparations from rats, mice and monkeys.<br>The panel cited the following as the sources of the information:<br>FASEB (1976) and Litton Bionetics (1976) |
| **Reliability** | : | (4) not assignable<br>Information taken from a review report. No actual data are given. |

03.12.2004 (6) (15)

## 5.7 CARCINOGENICITY

| | | |
|---|---|---|
| **Species** | : | Mouse |
| **Sex** | : | Male/female |
| **Strain** | : | C3H |
| **Route of admin.** | : | Dermal |
| **Exposure period** | : | 104 weeks |
| **Frequency of treatm.** | : | Twice weekly for 104 weeks |
| **Doses** | : | 50 mg/application |
| **Result** | : | Negative |
| **Control group** | : | yes |
| **GLP** | : | Yes |
| **Test substance** | : | PARL-3093-GR-81 |
| **Method** | : | 50 mg undiluted test material was applied twice weekly to the shorn interscapular region of 50 male and 50 female C3H mice aged 6-8 weeks. Positive control groups of 50 mice of each sex had 50 mg of a 0.05% solution of BaP in toluene applied twice weekly and these groups served as the positive controls. In addition solvent control groups of 50 mice of each sex received twice weekly applications of 50 mg toluene and a further group of 50 mice of each sex were untreated. The latter groups comprised the solvent and untreated controls respectively.<br><br>Applications were continued for 104 weeks or until a horny lesion on the surface of the skin grew to 1 $mm^3$. The lesion was diagnosed as a papilloma and the week that it appeared was recorded. If the tumor grew rapidly, invaded surrounding tissues, or became ulcerated and/or necrotic, it was diagnosed as an "advanced tumor" and the week of the transition was recorded. If a tumor regressed, treatment was resumed and continued until the end of the study or until another papilloma developed. If no growth appeared before death, the animal was recroded as not developing a tumor. If however, a second neoplasm developed, the time of its appearance was used in the calculation of the average latency period for the group. |

Animals were observed daily throughout the study for clinical signs of toxicity.
At the termination of treatment, all surviving animals were sacrificed. A complete post mortem examination was carried out on all animals sacrificed at the end of the study and on all animals that either died or were killed during the study because they were moribund.
At the post mortem examination the size and location of all skin neoplasms was recorded. Skin including the neoplasms and any other lesions was removed and placed in fixative for subsequent histopathological examination. Subcutaneous lymph nodes from the neck, ancillary region and groin areas were also removed from the same animals and prepared for subsequent microscopic examination. The chest, abdominal and cranial cavities were examined and all organs were removed and a note made of their gross appearance. Tissues from each organ were preserved for possible microscopic examination.
H & E sections of the skin and of the mammary glands were examined microscopically.

**Result** : The number of mice with histologically-confirmed tumors is shown in the following table.

| No. Mice | No. mice with tumors Malignant | Benign | Latent period (weeks) |
|---|---|---|---|
| Untreated controls | | | |
| 46 males | 0 | 0 | - |
| 50 females | 1 | 2 | - |
| Toluene controls | | | |
| 48 males | 3 | 3 | 87 |
| 50 females | 5 | 2 | 72 |
| Grease | | | |
| 47 males | 0 | 2 | 67 |
| 50 females | 1 | 0 | 82 |
| BaP | | | |
| 46 males | 21 | 5 | 48 |
| 49 females | 45 | 2 | 49 |

It was concluded that the test material was not a skin carcinogen.

**Test substance** : PARL-3093-GR-81 is a Lithium complex grease with the following composition

| | | |
|---|---|---|
| Base oil | approx | 80% wt |
| Li 12-hydroxystearate | | 7.5% wt |
| Other additives | approx | 12% wt |

**Reliability** : (2) valid with restrictions
It should be noted that this study was a study of skin carcinogenicity only.

03.12.2004 (3)

### 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

**Species** : Rabbit
**Sex** : Female
**Route of admin.** : Gavage
**Frequency of treatm.** : Single dose given
**Doses** : 2.5 mg/kg
**Result** : Negative
**Year** : 1967

**GLP** : No
**Test substance** : Vehicle containing 5.5% Magnesium stearate

**Result** : The CIR report states:
Fourteen females received the vehicle per os at a dose of 2.5 mg/kg 70 hours post coitus whereas 13 females were given the same dose 192 hours post coitus. Compared with anomalies in the fetuses from 16 untreated mothers (12 of 112 offspring had anomalies) the vehicle containing 5.5% magnesium stearate induced anomalies in 9 out of 86 and 11 out of 90 fetuses respectively, thus demonstrating the absence of teratogenic effect.
**Source** : Cosmetic Ingredient Panel review (1982)
**Test substance** : The test substance was a vehicle used to coat pharmaceutical tablets. The coating had the following composition:

| | |
|---|---|
| Polyethylene glycol | 27.5 mg |
| Starch | 34 mg |
| Talc | 27.5 mg |
| Silicon dioxide | 5.5 mg |
| Magnesium stearate | 5.5 mg |

**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel. The material tested contained only 5.5% magnesium stearate and the method was inadequate for an evaluation of developmental toxicity.

03.12.2004 (7)

**Species** : Various
**Remark** : Leonard et al reviewed information on the teratogenic effect of lithium compounds.
They comment that results have varied in intact animals.
Whereas some authors have not demonstrated teratogenic effcects of lithium compounds, others have done so. The malformations reported have included reduced number and weight of the litter, more resorptions, wavy ribs and incomplete ossification.
These discrepancies might be due to a different sensitivity of the species and strains used, the stress of daily injections and/or differences in lithium concentrations present in serum during critical periods of development.

Lithium carbonate given to mice over several days yielding serum levels comparable to those in man treated for manic-depressive disorders did not show any effects, but six times higher doses caused malformations in the offspring.
Chronic exposure to lithium at doses that produced serum levels of the same order as seen in patients was toxic but did not affect the entire litter nor was it teratogenic to individual embryos.

Many authors have reported that lithium causes congenital defects, especially of the cardiovascular system when given to women during the first trimester of pregnancy. As a result registers of "Lithium babies" have been set up. Up till now, analysis of the limited data have demonstrated an effect.
The authors conclude that the question of the possible teratogenicity of lithium remains open until further work is done.
**Reliability** : (4) not assignable
24.12.2003 (14)

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